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13. ABSTRACT *(Maximum 200 Words)*

Background: The role of lung inflammatory mediators in the development of lung injury following smoke inhalation is unknown.

Objectives: To evaluate the predictive value and role of inflammatory mediators in acute lung injury following smoke inhalation.

Specific aims: 1) Determine the predictive value of initial inflammatory markers in bronchial secretions of smoke inhalation victims for subsequent lung injury.
2) Measure longitudinal changes in inflammatory mediators in smoke inhalation victims.

Study design: Bronchial secretions from 200-250 intubated patients with smoke inhalation injury will be evaluated for initial (2 hours following cessation of exposure) and longitudinal changes (every two hours to a maximum of 72 hours) concentrations of substance P, TNF-α, IL-1, IL-8, and IL-10, as well as cell count and differential. Initial lung inflammation and changes in inflammatory markers will be compared in patients without and without subsequent significant lung injury.

Progress to date: We obtained IRB approval from the Army, the University of Arizona and the Arizona Burn Center, a process which continued through earlier this year. We tested and finalized protocols for sputum collection, storage and analysis. We are waiting for the first subjects to be enrolled in the study, which should occur in the immediate future.

14. SUBJECT TERMS
smoke inhalation, acute lung injury, inflammation, cytokines

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INTRODUCTION

The goal of this research is to identify inflammatory mediators playing key roles in acute lung injury (ALI) following smoke exposure. Our objectives are to determine the value of initial concentrations of these mediators in predicting later development of ALI, and to determine how the mediator concentrations change over time, which may also have predictive value and improve our understanding of the mechanism of smoke injury. We hypothesize that smoke inhalation results in rapid changes (within two hours) in lung inflammatory mediators, initial changes in lung inflammatory mediators are predictive of the extent of subsequent lung injury and changes over time in lung inflammatory mediators will precede clinical findings of acute lung injury. Over the three remaining years of this grant, we will be evaluating initial concentrations and changes over time of inflammatory mediators in pulmonary secretions of approximately 200 ventilated patients with smoke inhalation. The clinical course of these patients will be tracked, including % body surface area burn, days on a ventilator, days in ICU, pulmonary infiltrates, white blood cell count, fever, sputum volume, oxygen requirements, blood oxygenation, and development of ALI.

BODY

The major activity of the first year of this research has been to obtain Institutional Review Board (IRB) approval from the Army, the University of Arizona, and the Maricopa Integrated Health System (MIHS), which is the parent institution of the Arizona Burn Center where the subjects will be enrolled in the study and the bronchial suction material and clinical outcome data collected. This process took much longer than anticipated. A primary difficulty which has now been overcome was the issue of "intent to benefit" which is required for Army approval and must accrue to the subject, and which differs from non-Army IRBs where benefit to society is adequate, as long as there is no potential harm to the subject. After extensive review and revision of the study protocols, including the mailing of fire prevention materials to subjects after hospital discharge, it was possible to obtain the Army IRB approval. MIHS also had a newly approved IRB, and additional time was needed to satisfy their needs while at the same time satisfying the University of Arizona IRB board. Our final consent forms now have stamps from both of the Arizona IRBs. We are now ready to enroll subjects in the study and expect in the immediate future (truly any day) smoke inhalation victims to be admitted to the Arizona Burn Center, given the annual census of 80-100 inhalation victims each year over the last several years.

The other major projects in preparation for the study were to generate data collection forms (please see appendices) and to determine the best means to preserve bronchial suction samples. Since inhalation injury patients are suctioned every two hours while intubated, and we will be collecting these samples for up to 72 hours after injury, it is necessary to have the respiratory therapists in the Arizona Burn Center collect the samples. It is not financially feasible to have a student or other research assistant potentially available 24 hours a day 7 days a week. Since the respiratory therapists have clinical duties, the initial sample collection must be rapid and not complex.

After extensive evaluation, we chose to have 500 ul of the bronchial suction material aliquoted into an equal volume of methanol. We have found that this process maintains our ability to perform cell counts and differentials for up to a week following collection. This allows for samples to be picked up at the Arizona Burn Center on a weekly basis and then transported to the University of Arizona in Phoenix.

We have also evaluated various means of processing and storing the bulk of each bronchial suction sample for later analysis of cytokines. This is necessitated by the use of 96 well ELISA plates for cytokine analysis which requires collection of up to 40 samples (run in duplicate and allowing for the standard curve wells) for efficient use of resources. Comparison of freshly processed samples, rapidly frozen (at -80°C) and methanol preserved samples demonstrated that rapid freezing of samples caused the least disruption in subsequent measurement of cytokines in supernatant.

The initial research proposal mentioned linkages with another investigator at the University of Arizona who had submitted a proposal to perform similar work on an animal model to the U.S. Army Medical Research and Materiel Command. Since this other proposal was not funded, we have been investigating the possibility of collaborating with other investigators with experience in animal models of smoke exposure. Fortunately, Dr. Edgar Kimmel of the Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL) has agreed to collaborate on developing an animal model that will reflect the findings that we will measure in humans. This collaboration will assist in the application for additional funds for this research, and will be essential in the future testing of pharmacologic therapies for smoke inhalation.

KEY RESEARCH ACCOMPLISHMENTS

Since no subjects have been enrolled in the study, it is not yet possible to present key research findings concerning the two specific aims of the study. For sample collection and analysis, we have completed the following accomplishments, which are important in that they permit more efficient and less costly mechanisms of addressing the specific aims of the study.

- Selection of methanol as a preservative to permit analysis of a small aliquot of bronchial suction material cell count and differential up to one week following collection, based on direct comparison with other preservatives and directly processed samples without preservation.
- Selection of rapid freezing of the majority of bronchial suction material to permit later processing for supernatant analysis of cytokines, based on direct comparison of ELISA cytokine measurements in fresh samples, rapidly frozen samples, and use of other preservatives.
- Generation of data entry forms

REPORTABLE OUTCOMES

We have initiated collaboration with Dr. Edgar Kimmel of the Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL), who has extensive experience with animal models of smoke inhalation injury, particularly from combustion of composite materials. The objectives of this collaboration are to develop small animal models of smoke inhalation injury that will yield results similar to those seen in human smoke inhalation victims. This will permit us to further study mechanisms of smoke inhalation injury and to test new potential treatment regimens.

CONCLUSIONS

We have determined that preserving a portion of the bronchial suction material supernatant in methanol permits analysis of cell count and differential for up to 7 days following collection. We have also determined that freezing the bronchial suction material does not significantly change the cytokine measurements as performed by ELISA. We have initiated collaboration with the Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL) with the objective of finding animal models of smoke inhalation injury that will yield results similar to those seen in human smoke inhalation victims.

REFERENCES

None

APPENDICES (see following pages)

1) Memorandum from Edgar C. Kimmel, PhD, Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL)

2) Data entry forms

MEMORANDUM

To: Jeffery L. Burgess, MD, MPH
Environmental and Community Health
College of Public Health, University of Arizona

From: Edgar C. Kimmel, Ph.D.
Head Inhalation/Pulmonary Effects Laboratory
Geo Centers, Inc at the
Naval Health Research Center/ Environmental Health Effects Laboratory (NHRC/EHEL)

CC: CDR Warren W. Jederberg, MSN, USN,
Officer-in-Charge, NHRC/EHEL

Subject: Collaborative investigation of biomarkers of Acute Lung Injury following smoke inhalation
Date: 8 May 2003

Dr. Burgess we are enthusiastic over the prospect of developing a collaborative research directed toward identifying predictive biomarkers of Acute Lung Injury (ALI) Acute Respiratory Distress Syndrome (ARDS) following inhalation of smoke. The research aims and objectives spelled out in your project entitled "Acute Lung Injury Following Smoke Inhalation: Predictive Value of Sputum Biomarkers and Time Course of Lung Inflammation" are practically identical to those which guide our smoke induced ALI/ARDS research program. The fundamental difference between these two research efforts being, of course, that your efforts involve examination smoke inhalation victims directly while our efforts involve the development of small animal models of smoke induced ALI/ARDS. Our objectives also are to clarify the role of specific mediators of the inflammatory response in the pathogenesis of ALI/ARDS with the hopes of identifying biomarkers of prognosis in smoke victims as well as identifying points of intervention. We also believe that a well characterized dose/response related animal model of ALI/ARDS can be used to systematically evaluate proposed new treatment regimens that require experimental validation prior to implementation as treatment for smoke inhalation victims.

Or laboratory, NHRC/EHEL, has a history of successful research of this nature as demonstrated by our efforts to characterize acute toxicity and pulmonary responses to smoke from advanced composite materials used in the manufacture of military and civilian aircraft.

It is our opinion that collateral research in animal models and in human smoke victims can be used to exploit the advantages of both resulting in a more comprehensive understanding of the pathogenesis and mechanisms of smoke related ALI/ARDS that will lead to more efficacious treatment of this syndrome. Therefore we are delighted to have the opportunity to develop collaborative research programs with your laboratory.

Respectfully,
E.C. Kimmel

ID LABEL: "WBC & Rx"

WBC COUNTS								
DATE								
TOTAL								
DIFFERENTIAL								

Medications				Blood or Plasma Product transfusion Record		
Name	**Dosage**	**Start Date**	**Stop Date**	**Date**	**Time**	**Quantity**

Other Comments:

GENERAL ID LABEL
"ICU-VENT"

SERIAL LABELS	Date in mm/dd/yy			Time on 24 hr scale		FiO2	PEEP	Tidal Volume	Resp Rate	Minute Ventillation	Max Airway Press.	N.Saline Injected	Frozen samp vol	Meth samp vol	Tech Name
Serial label 1															
Serial label 2															
Serial label 3															
Serial label 4															
Serial label 5															
Serial label 6															
Serial label 7															
Serial label 8															
Serial label 9															
Serial label 10															
Serial label 11															
Serial label 12															
Serial labels	Date in mm/dd/yy form			Time in 24 hr form		FiO2	PEEP	Tidal Volume	Resp Rate	Minute Ventillation	Max Airway	N.Saline Injected	Frozen samp	Meth samp	Tech Name

										Press.		vol	vol	
Serial label 13															
Serial label 14															
Serial label 15															
Serial label 16															
Serial label 17															
Serial label 18															
Serial label 19															
Serial label 20															
Serial label 21															
Serial label 22															
Serial label 23															
Serial label 24															
Serial label 25															
Serial labels	Date in mm/dd/yy form			Time in 24 hr form		FiO2	PEEP	Tidal Volume	Resp Rate	Minute Ventillation	Max Airway Press.	N.Saline Injected	Frozen samp vol	Meth samp vol	Tech Name
Serial label 26															

Serial label 27														
Serial label 28														
Serial label 29														
Serial label 30														
Serial label 31														
Serial label 32														
Serial label 33														
Serial label 34														
Serial label 35														
Serial label 36														

SMOKE INHALATION STUDY

NAME	MARICOPA MEDICAL CENTER MEDICAL RECORD NUMBER
FIRST _____ MIDDLE _____ LAST _____	

Study ID

Put ID label: **STUDY ID**

PERMANENT ADDRESS

CITY | STATE | ZIP

ID LABEL:SingleMp1

SINGLE MEASURE DATA SHEET

DATE OF BIRTH ____/____/____ mm/dd/yy		DATE OF EXPOSURE	___/___/___ mm/dd/yy
GENDER **male** **female**		TIME OF EXPOSURE	24 hr scale
PERCENTAGE BODY SURFACE AREA BURNT **PARTIAL**_____% **FULL THICKNESS**_____%		DATE OF ADMISSION	___/___/___ mm/dd/yy
WEIGHT _____ lbs HEIGHT _____ cms		TIME OF ADMISSION	24 hr sclae
DATE INTUBATED	___/___/___ mm/dd/yy	DATE EXTUBATED	___/___/___ mm/dd/yy
TIME INTUBATED	24 hr scale	TIME EXTUBATED	24 hr scale

ASSOCIATED TRAUMA YES NO *IF YES THEN DESCRIBE*

ASSOCIATED FRACTURES YES NO *IF YES THEN DESCRIBE*

ORGAN FAILURE PRESENT YES NO **IF YES** THEN DESCRIBE

ID Label: Single Mp2

SEPSIS PRESENT	YES	NO	**IF YES THEN BLOOD CULTURE**	POSITIVE NEGATIVE
OTHER CRITERIA **COMMENTS**				

BRONCHOSCOPY PERFORMED yes no	
IF YES THEN DATE: ___/___/___ mm/dd/yy	**SEVERITY SCALE** **1** **2** **3** **4** **5**
RESULTS:	

PAST HISTORY

COPD	YES	NO	If yes then add comments if required
Asthma	YES	NO	If yes then add comments if required
Liver disease	YES	NO	If yes then add comments if required
Diabetes Mellitus	YES	NO	If yes then add comments if required

GENERAL ID LABEL "ABG"

ABG RESULTS

Date in mm/dd/yy	Time on 24 hr scale	pH	PaO_2	O_2 saturation	$PaCO_2$	Base Excess

General id label: "CXR"

CHEST X-RAY FINDINGS
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS:
DATE: FINDINGS: